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Published in:
Analytical Chemistry

DOI:
[10.1021/ac001428j](https://doi.org/10.1021/ac001428j)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2001

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

van Trigt, R., Kerstel, E. R. T., Visser, GH., & Meijer, H. A. J. (2001). Stable isotope ratio measurements on highly enriched water samples by means of laser spectrometry. *Analytical Chemistry*, 73(11), 2445-2452. <https://doi.org/10.1021/ac001428j>

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Stable Isotope Ratio Measurements on Highly Enriched Water Samples by Means of Laser Spectrometry

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We demonstrate the feasibility of using laser spectrometry (LS) to analyze isotopically highly enriched water samples (i.e., $\delta^2\text{H} \leq 15\ 000\text{‰}$, $\delta^{18}\text{O} \leq 1200\text{‰}$), as often used in the biomedical doubly labeled water (DLW) method to quantify energy metabolism. The method is an important extension of a recently developed infrared laser direct absorption spectrometer. The measurements on highly enriched, small-size (10 μL liquid water) samples show a clearly better accuracy for the $^2\text{H}/^1\text{H}$ ratio. In the case of $^{18}\text{O}/^{16}\text{O}$, the same level of accuracy is obtained as with conventional isotope ratio mass spectrometer (IRMS) analysis. With LS, the precision is better for both $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$. New is the ability to measure $^{17}\text{O}/^{16}\text{O}$ with the same accuracy as $^{18}\text{O}/^{16}\text{O}$. A major advantage of the present technique is the absence of chemical sample preparation. The method is proven to be reliable and accurate and is ready to be used in many biomedical applications.

Stable and radioactive isotopes are almost ideal tracers in a wide variety of fields, such as glaciology, hydrology, atmospheric and climate research, medicine, and biology.^{1–5} In biology, a well-known and often-used application of stable isotopes is the doubly labeled water (DLW) method, which quantifies the energy expenditure of nonrestrained humans as well as free-living animals by the level of CO_2 production.^{6–8} To this end, a known amount

of water, enriched in both ^{18}O and ^2H , is administered to the subject, either orally, intraperitoneally, subcutaneously, or intravenously. After a short equilibration period, an initial blood, saliva, or urine sample is taken. The enrichment of the body water pool as a result of the administration of DLW enables the calculation of the size of the body water pool. After some time, typically 24 h, a final sample is taken. The final sample will show lower ^{18}O and ^2H concentrations than the initial. The lower ^{18}O concentration of the final sample is caused by the CO_2 and H_2O turnover during the measurement interval. The H_2O turnover is independently monitored by the ^2H concentration such that in the end, the CO_2 production can be calculated from the difference in ^{18}O and ^2H turnover rates. Thus, at a known diet, the energy expenditure of the animal is known.^{5–9}

It is common practice to report the isotope ratio, R , not in absolute terms, but rather as the deviation of the abundance ratio of the sample with respect to the same abundance ratio of a calibration material. The internationally accepted calibration material for water is Vienna Standard Mean Ocean Water (VSMOW).⁹ Thus, isotope measurements are generally reported as

$$\delta(x) = \frac{^xR_{\text{sample}}}{^xR_{\text{VSMOW}}} - 1 \quad (1)$$

where x represents the rare isotopomer. Because the values of δ are generally small, they are given in per mil (‰). Furthermore, one needs to realize that the current experiment does not measure the atomic concentration ratio (e.g., $^{18}\text{O}/^{16}\text{O}$), but rather, the molecular ratio (in this case: $^{1}\text{H}^{18}\text{O}^1\text{H}/^{1}\text{H}^{16}\text{O}^1\text{H}$). However, for all practical applications, the molecular and the atomic δ -values are indistinguishable, principally because the ^2H , ^{17}O and ^{18}O isotope abundances are very small. Thus,

$$\delta(^1\text{H}^{18}\text{O}^1\text{H}) = \delta^{18}\text{O}, \delta(^1\text{H}^{17}\text{O}^1\text{H}) = \delta^{17}\text{O} \text{ and } \delta(^2\text{H}^{16}\text{O}^1\text{H}) = \delta^2\text{H}$$

The traditional method of measuring isotope ratios makes use of an isotope ratio mass spectrometer (IRMS), which is specially designed for this purpose. IRMS, however, is not capable of

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measuring condensable gases, such as water, directly, thus making extensive sample preparations necessary. These usually involve reduction of water to H_2 gas over hot zinc¹⁰ or uranium¹¹ in order to determine $\delta^2\text{H}$, and equilibration of water with CO_2 at a well-controlled temperature to determine $\delta^{18}\text{O}$.¹² In many laboratories, both procedures are performed on a routine basis, but they can introduce systematic and accidental errors,¹³ are time-consuming, and in the case of deuterium, potentially hazardous. It is, therefore, no surprise that many efforts have been and are being undertaken that are aimed at eliminating one or more of these drawbacks. Recent examples are the development of continuous flow isotope ratio mass spectrometry (CF-IRMS) techniques¹⁴ coupled with an on-line reduction system using manganese,¹⁵ copper,¹⁶ or chromium.¹⁷ As an alternative off-line preparation method for ^2H , lithium aluminum hydride has been used.¹⁸

Recently, we reported on a new technique based on laser spectrometry (LS) to measure the isotope ratios in water at natural abundance levels, that is, with isotope ratios between those of VSMOW and the other international calibration material, Standard Light Antarctic Precipitation (SLAP).¹⁹ Our method is based on absorption changes in the low-pressure, gas-phase, infrared spectrum of a water sample as induced by changes in the isotope concentrations. At sufficiently low pressure, the infrared absorption spectrum of water shows well-resolved individual rovibrational transitions ("lines") that can be uniquely identified with the various isotopomers of the water molecule. The absorption strength of such a line is linearly dependent on the concentration of the associated isotopomer. (Note that the absorption strength is simply calculated from the measured absorption or light attenuation by means of the Beer–Lambert law). We have identified a specific part of the water spectrum where all four of the isotopomers of interest (i.e., H^{18}OH , H^{17}OH , H^{16}OH and $^2\text{H}^{16}\text{OH}$) appear near to each other (but are not overlapping) and are at intensities that are comparable to natural abundances. Comparison of the line intensities in the spectrum of a sample water with those of the simultaneously measured reference water directly gives the relative isotope ratio or δ -value.

In this study, we report on our efforts to extend the LS method to the range of isotopically enriched waters encountered in biomedical applications of the DLW method ($\delta^2\text{H} \leq 15\ 000\text{‰}$, and $\delta^{18}\text{O} \leq 1200\text{‰}$). These high enrichments are common in energy expenditure measurements on small mammals and birds, which generally show a large water turnover and high energy metabolism, resulting in typical ^{18}O half-times of 8–12 h. Because it is

usually necessary to observe the animal's day–night cycle, measurements are taken over a period of 24 h, or 3–4 ^{18}O half-times. During the breeding season, when parents do not visit their offspring every day, the measurement period may have to be extended to 48 h, or 6–8 half-times, yielding correspondingly low final enrichment values. Because the final value should not be too close to the predose background for accuracy reasons, the initials are necessarily (very) high. In addition, one cannot tolerate large sample sizes, in contrast to the case of energy expenditure studies on large animals or humans. Therefore, the DLW application discussed in this paper is clearly a highly demanding one in terms of isotope ratio measurements.

As we will demonstrate, the laser-based method has some important advantages over the alternatives mentioned earlier and is highly competitive with the traditional methods, particularly in cases as outlined above.

EXPERIMENTAL SECTION

In the following section we will first discuss the preparation of the standards that are used for calibration purposes, as well as the unknown samples used in this comparative study. Subsequently, we will describe the experimental procedures and techniques for the isotope measurements.

Standards. The only reliable way of obtaining "absolute" isotope standards is by gravimetric methods. For ^2H , accurate gravimetric preparation of standards is possible, thanks to the fact that isotopically pure $^2\text{HO}^2\text{H}$ and $^1\text{HO}^1\text{H}$ are readily available. In fact, the $^2\text{H}/^1\text{H}$ abundance ratio of the calibration materials VSMOW and SLAP are known absolutely by way of gravimetric mixing.^{20–22}

For ^{18}O and ^{17}O , the situation is not so simple. Neither is it possible to obtain 100% pure ^{16}O , ^{17}O , or ^{18}O containing water, nor it is possible to know the isotope composition with a high degree of accuracy, although some efforts toward this goal have been published.^{23–26} It is possible, however, to construct a dilution series of working standards while maintaining a well-known, linear relation between the enrichment levels of the different isotopes. We prepared our working standards for this study by gravimetric mixing of a distilled water with a certified heavily enriched water ($^{18}\text{O} = 94.5$ and $^{17}\text{O} = 19.2$ atom %) and almost pure D_2O ($^2\text{H} = 99.9$ atom %) and using a calibrated balance (Sartorius Analytic). The independent ^{17}O enrichment of the standards is a novelty, required here to test the unique capability of our LS system to measure $\delta^{17}\text{O}$ in addition to the usual $\delta^{18}\text{O}$ and $\delta^2\text{H}$ (see ref 19). A range of enrichments was created from one mother mixture to avoid an accumulation of errors. The weighing uncertainties yield uncertainties for the linearity of the isotope ratio scale that are in all cases smaller than the measurement accuracy of either the IRMS or the LS instrumentation (see Table 1). We will come back to this point in the discussion.

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Table 1. Calculated Values of the Gravimetrically Mixed Enriched Standards^a

	%o		
	$\delta^2\text{H}$	$\delta^{17}\text{O}$	$\delta^{18}\text{O}$
TLW-0	-41 (1)	-3.1 (1)	-6.3 (1)
TLW-1	1273 (10)	28.9 (6)	97.8 (5)
TLW-2	2585 (20)	60.9 (10)	201.8 (18)
TLW-3	5217 (50)	125.1 (20)	410.3 (20)
TLW-4	10 820 (100)	261.7 (40)	854.3 (40)

^a The values rely on the specified enrichments of the commercial starting material. Errors are worst case estimates of the effect of weighing uncertainty in the mixing process and are given in units of the least significant digit.

Unknown Samples. As unknown samples, we used 51 vials of blood of Japanese quails (*Coturnix c. japonica*) obtained in a validation study of the DLW method against respiration gas analysis.²⁷ All of the blood samples were distilled on a micro-distillation column. Among the samples were backgrounds, taken prior to the administration of enriched doubly labeled water, initials with expected values of $\delta^2\text{H} \leq 15\text{‰}$, $\delta^{18}\text{O} \leq 1200\text{‰}$, and $\delta^{17}\text{O} \leq 350\text{‰}$, and finals with isotope enrichments between the initial and background values.

Isotope Measurements. We measured all of the samples using both IRMS and LS. Samples were regularly alternated with our working standards in order to calibrate the instruments and check their performance. The order of the measurement of samples and working standards in both systems was determined such that large steps in enrichment (read: memory effects) were avoided. The IRMS measurements were carried out in four short periods (5–10 days) between February and July, 2000. The LS measurements were carried out in 16 days in July, 2000.

Procedures. Isotope Ratio Mass Spectrometry. All of the samples were prepared and measured at the Centre for Isotope Research (CIO) using routine procedures and standard equipment. For each water sample, four glass microcapillary tubes were filled, each containing between 10 and 15 μL of water. The capillaries were flame-sealed immediately after filling. The use of these capillaries was dictated by the available instrumentation and was in no way essential to the method. To obtain the isotope ratios, the capillary tube was put in an on-line vacuum distillation system, mechanically broken, and cryogenically frozen into a quartz vial. The Epstein–Mayeda equilibration method¹² was used to determine $\delta^{18}\text{O}$ of the samples: 2 mL of CO_2 gas of known isotopic composition was added to the vial, which was subsequently kept in a thermostated water bath at 25 °C for at least 48 h. After this, the isotopically equilibrated CO_2 was removed for IRMS analysis and the remaining water was led over a uranium oven at 800 °C to produce H_2 .¹¹ The $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ isotope ratios of the CO_2 and the H_2 gases, respectively, were determined using dual-inlet isotope-ratio mass spectrometers: a Micromass SIRA 10 for CO_2 and a SIRA 9 for H_2 . In this way, we obtained four independent isotope ratio determinations for both isotopes and for each sample.

Laser Spectrometry. A detailed description of the LS method is available elsewhere.^{19,28} In brief, we measured the gas-phase direct

absorption spectrum from a water sample in the 2.7- μm region, determined the strength of the absorption of the different isotopomers, and compared these to the absorption strengths of a simultaneously recorded reference water spectrum. To record these spectra, a single mode Color Centre Laser (Burleigh) was scanned over the range from 3664.05 to 3662.70 cm^{-1} in about 2500 steps. During the scan, both the laser power after passage through the gas cells containing the water vapor and the laser power before the cells was measured using phase-sensitive detection with amplitude modulation at $\sim 1\text{ kHz}$. Currently we have four gas cells available. These are equipped with multiple pass optics to achieve an optical path length of $\sim 20\text{ m}$. The cells are made of stainless steel (mirror holders) and a glass tube; their volume is $\sim 1\text{ l}$. They show a memory effect (i.e., contamination with previously measured water) that amounts to up to about 5% of the difference in enrichment levels between two samples. This implies that generally, the first measurement after a large step in enrichment (for example, 2000‰ for $\delta^2\text{H}$ and 300‰ for $\delta^{18}\text{O}$) must be discarded. We tried to avoid such large enrichment steps by taking care of the sample injection order; to this end we used the expected values from the biomedical experiment, in agreement with common IRMS procedures (where the ^2H preparation system produces even larger memory effects; see Calibration). The glass tube of the cell is equipped with a valve that has a small (1-mL) chamber behind it, the injection chamber. The injection procedure was the following: After removal of the previous sample by evacuating the cells, we flushed all four of the cells simultaneously with dry nitrogen gas. Cross-contamination between the cells was avoided by cryogenic traps between each gas cell and the vacuum pump. After filling the cells with 1 atm of nitrogen gas, the injection chambers were closed. The cells were then evacuated again, while in the meantime, we injected 10 μL of liquid water samples with syringes through rubber septa into all four of the injection chambers. After closing the main pump valves, the injection chambers were opened and the water was evaporated, along with the nitrogen, into the main volume of the cells. The final pressure was $\sim 13\text{ mbar}$, well below the saturation vapor pressure of water at room temperature. The laser started scanning after a 5-min waiting period to ensure that all of the water had evaporated. The entire sample introduction procedure took 15 min. One gas cell was reserved for the reference water; of the other cells, one contained a working standard (thus, giving us a permanent check against standards over the entire measurement period), and the two remaining cells contained unknown water samples. As an extra precaution, the reference was treated in the same manner as the samples and refreshed after every measurement to ensure its isotope ratio could not change as a result of slow mixing with external water or isotope fractionation effects. The infrared absorption spectra of the waters injected into the four gas cells were measured simultaneously. For each injection, 12 successive scans were recorded, each taking about two min. A full measurement, including injection and removal of the sample, takes $\sim 40\text{ min}$. The sample throughput for the LS is, thus, currently ~ 4 measurements (samples and/or working standards) per hour. All of the samples and standards were injected and measured at least five times to collect some statistical data and to be able to remove measurements affected by memory effects. The exact procedure

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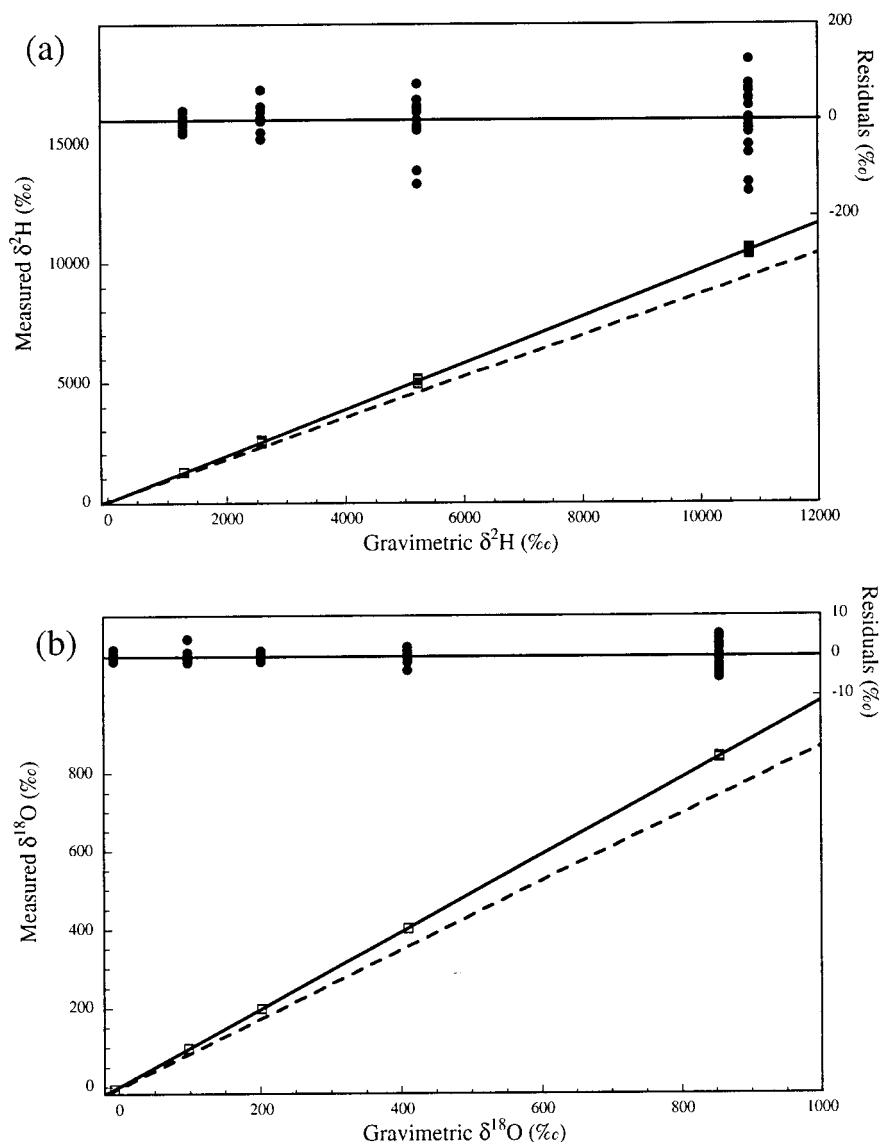


Figure 1. Squares represent the (a) $\delta^2\text{H}$ and (b) $\delta^{18}\text{O}$ IRMS measurements after application of the known corrections. The solid line is the normalization curve obtained in a linear regression analysis. Also shown are the residuals (measured value minus fit). The broken line is a least-squares fit to the raw measurements.

for calculating the raw, uncalibrated, δ -values from the recorded spectra is straightforward and is described elsewhere.²⁸

Calibration. *Isotope Ratio Mass Spectrometry.* Calibration for both of the IRMS machines was maintained by daily tests with local reference gases (one at natural abundance, the other enriched) as well as with several local water standards, in addition to the standards that were specific for this project. For H_2 , the H_3^+ -correction was measured on a daily basis and in the current range amounted to $\leq 12\%$ of the value measured. Further, a correction for cross-contamination $\leq 0.5\%$ of the value was applied, as described previously.²⁹ Both of these effects are thought to be well-understood and can be quantified independently. Therefore, these corrections, together with the conversion from machine reference gas to the VSMOW standard, were applied before the usual scale expansion correction (normalization). In the case of the oxygen isotope ratio, corrections were applied for cross-

contamination (smaller than 1%), and the water correction (for the amount of oxygen in the added CO_2 , causing dilution of the original oxygen in H_2O ; between 10 and 20%). Again, these corrections were applied before conversion to the VSMOW scale and the final scale expansion or normalization.

The scale expansion correction for the H_2 and CO_2 IRMS machines was similar to the one recommended by the IAEA for the natural range between SLAP and VSMOW;^{30,31} however, in the current enrichment range, the usual VSMOW–SLAP normalization would lead to a large, and inaccurate, extrapolation and was, therefore, not applied. Instead, we used our series of 5 gravimetrically determined standards to define the scale in a linear fit with equal weighting factors. Unfortunately, the $\delta^2\text{H}$ measurements involving the least enriched standard had to be rejected

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Table 2. Normalization Factors for IRMS and for the Different Sample Cells in the Case of LS^{a,b}

	IRMS	cell I	cell II	cell III
$\xi(\text{H}_2^{18}\text{O}) \cdot 10^2$	2.01 (8)	1.60 (7)	1.33 (7)	1.60 (9)
$\xi(\text{H}_2^{17}\text{O}) \cdot 10^2$	-	3.5 (2)	3.99 (2)	3.3 (2)
$\xi(^2\text{HOH}) \cdot 10^2$	3.2 (2)	1.3 (3)	0.5 (3)	0.2 (5)
$\psi(^2\text{HOH}) \cdot 10^3$		1.6 (3)	2.6 (3)	2.5 (4)

^a The errors between parentheses represent one standard deviation in units of the least significant digit. ^b $\delta_{\text{calibrated}} = (1 + \xi)\delta^* + \psi(\delta^*)^2$, with δ^* being the measurement value after initial corrections (see text). The quadratic term applies only to ^2HOH .

because of an excessive memory effect in the H_2 gas preparation system. Figure 1 shows the IRMS measurements before and after application of the known corrections mentioned earlier. The figure also gives the residuals of a linear regression analysis. The slope of this fit is the scale expansion factor, which is presented in Table 2.

Laser Spectrometry. In contrast to IRMS, LS does not require large corrections of the raw measurement values. The only correction applied before scale normalization was due to the effect on the final measurement of small pressure differences between the gas cells. This correction has been described in detail in the literature^{19,28} and, with proper sample introduction, amounts to no more than 2‰ and 6‰ in terms of the δ -values for the oxygen isotope ratios ($\delta^{17}\text{O}$ and $\delta^{18}\text{O}$) and $\delta^2\text{H}$, respectively. Note that this is much smaller ($\sim 0.1\%$) than the corrections that were applied in the mass spectrometer case. Again, the gravimetric working standards were used to determine the correct scale expansion factors, now also for $\delta^{17}\text{O}$. It turned out that for ^{17}O and ^{18}O , a linear normalization is sufficient, but for ^2H a second order correction was needed to reduce the residuals of the measurements at higher enrichments. The normalization factors for the three sample cells differed slightly. For all three of the measurement cells, the normalization plots and corresponding residuals are given in Figure 2. The scale expansion factors are listed in Table 2, together with the corresponding IRMS corrections.

RESULTS AND DISCUSSION

From Table 2 it is evident that IRMS requires a still substantial scale expansion. For both ^{18}O and ^2H , IRMS initially underestimates the true isotope ratios. The magnitude of the scale expansion factor found here in the high enrichment regime is similar to the one found in the natural isotope abundance range (VSMOW–SLAP normalization). Although this normalization has become common practice, the underlying physics is not understood. That no quadratic component is necessary to obtain a good fit in the normalization process may simply be due to the missing data at the lowest end of the scale.

Despite the very different and conceptually much simpler measurement technique, LS turns out to need a quantitatively similar normalization (see Table 2). Surprisingly, the scale expansion factor for ^{17}O is nearly twice as large as for ^{18}O , whereas the opposite might be expected if residual isotope fractionation effects were to blame.³² Moreover, fractionation effects are, in general, much larger for ^2H than for ^{18}O and certainly when

compared to ^{17}O , are in apparent contradiction to the data. Therefore, we strongly believe that the results indicate that our series of gravimetric standards contain 2% to 4% less ^{17}O than calculated from the specifications provided by the supplier of the starting material. To a lesser extent, the same may be true for ^{18}O . This should not surprise us, considering the difficulty in determining the absolute oxygen isotope concentrations (see Standards).

In any case, for the DLW application, the absolute value of the isotope ratios is not important: the calculated energy expenditure depends on the ratio of initial and final isotope concentrations (above background) and requires only a good linearity of the scale. The latter is assured by the calibration and normalization procedure carried out here.

The normalization factors of the sample gas cells are sensitive to the optical alignment causing small differences between the three sample gas cells. This is almost certainly due to residual Etalon fringes (interference effects) in the optical system that persist despite the use of antireflection-coated, wedged optics and careful alignment.

Accuracy. A good measure of the accuracy of the entire sample handling and measurement procedure is the root-mean-square (rms) value of the residuals of the standards (i.e., calibrated measurement value minus gravimetric value).

For the IRMS measurements on the working standards, the rms values of the residuals, as they appear in Figure 1, increase in size with enrichment. For $\delta^{18}\text{O}$, the values increase from $\sim 1\%$ to 3‰ over the range of enrichments studied here, whereas for $\delta^2\text{H}$, the rms values of the residuals increase from 17‰ to 68‰ (note that the measurement of the lowest enrichment standard was not included).

The rms values of the residuals of the LS measurements, as they appear in Figure 2, are also increasing in size with enrichment. Their values range from $\sim 1.5\%$ to 3.5‰ for ^{18}O , from 3‰ to 55‰ for ^2H , and from 1‰ to 2‰ for ^{17}O . Especially if one excludes the measurement at the highest enrichment level (which appears to break with the trend established at the lower enrichment levels), the LS performs significantly better for ^2H than IRMS.

For both IRMS and LS, all of the unknown samples are corrected and normalized as described for the standards.

In Figure 3 we directly compare IRMS and LS for all measured samples (standards and unknowns). From the preceding, it may be clear that over the range spanned by the standards, the two methods agree within their precisions. However, at the even higher enrichment levels encountered in the $\delta^2\text{H}$ measurements of the unknown samples, the LS method gives slightly higher values than does IRMS. This may indicate that IRMS, just as LS, needs a quadratic component in its normalization of the $\delta^2\text{H}$ scale in addition to the one already applied for cross-contamination.

Precision. The precision is given by the standard deviation (SD) of repeated measurements on the same sample (standards as well as unknowns). Their values increase with increasing enrichments, just as the rms values do. The SD of the IRMS measurements ranges from $\sim 1\%$ to 5‰ for $\delta^{18}\text{O}$, and from 5‰ to 100‰ for $\delta^2\text{H}$. For the LS measurements, the range for $\delta^{18}\text{O}$ is from 1‰ to 4‰ and for $\delta^2\text{H}$, from 5‰ to 60‰. LS can also measure $\delta^{17}\text{O}$, and its precision ranges from 1‰ to 2‰. These are essentially the same numbers as those obtained in the previous

(32) Meijer, H. A. J.; Li, W. J. *Isot. Environ. Health Stud.* **1998**, *34*, 349–369.

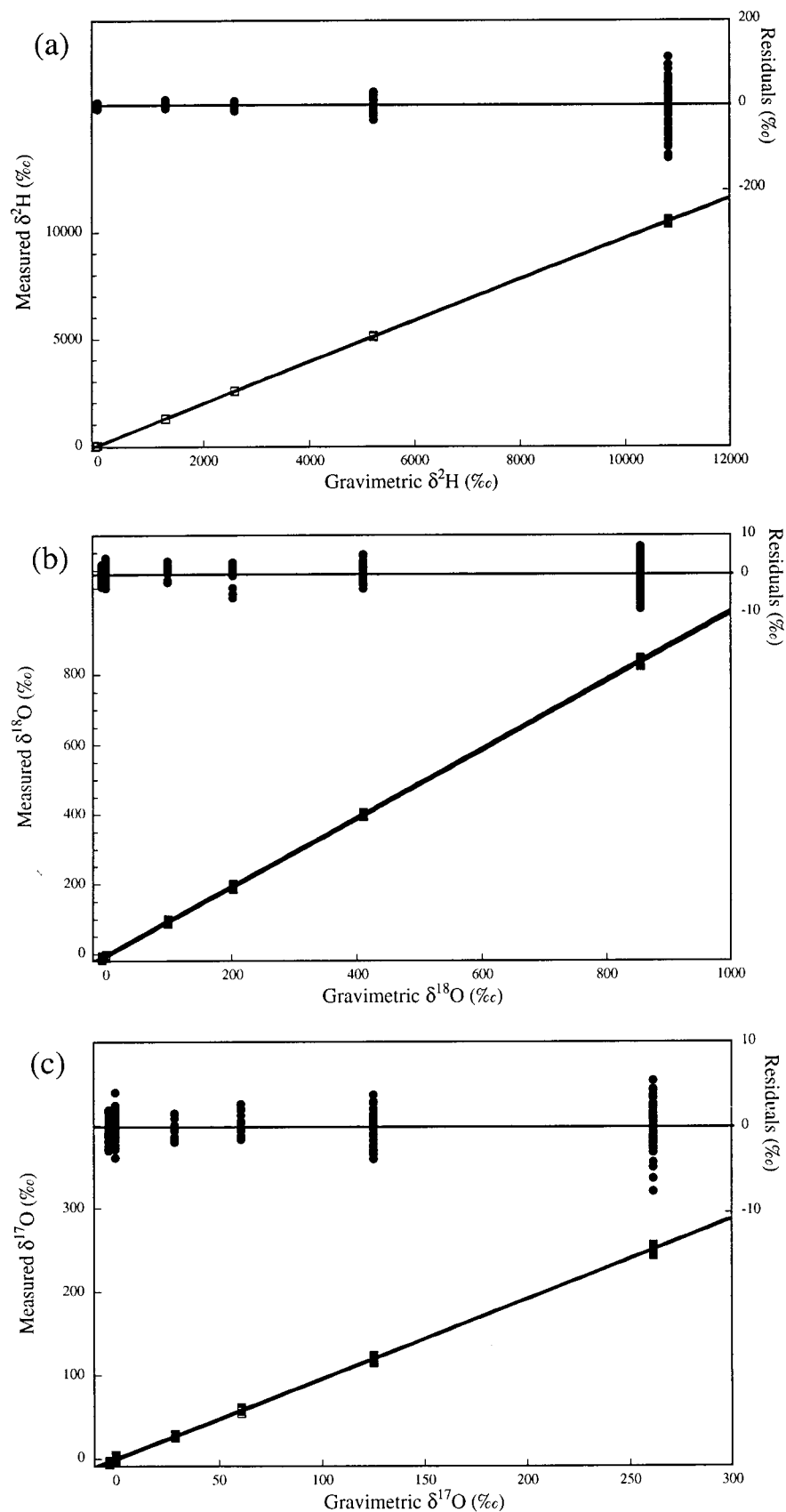


Figure 2. Squares represent the (a) $\delta^2\text{H}$, (b) $\delta^{18}\text{O}$, and (c) $\delta^{17}\text{O}$ LS measurements after application of the known differential pressure correction. The solid lines are the normalization curves obtained in a linear regression analysis (three; one for each sample cell, but overlapping at the current scale). Also shown are the residuals (measured value minus fit).

section for the accuracy, which indicates that the calibration procedure is not limiting the overall accuracy of the method.

Further Improvements. In principle, the ability to measure $\delta^{17}\text{O}$ with the LS system could be used to extend the DLW method

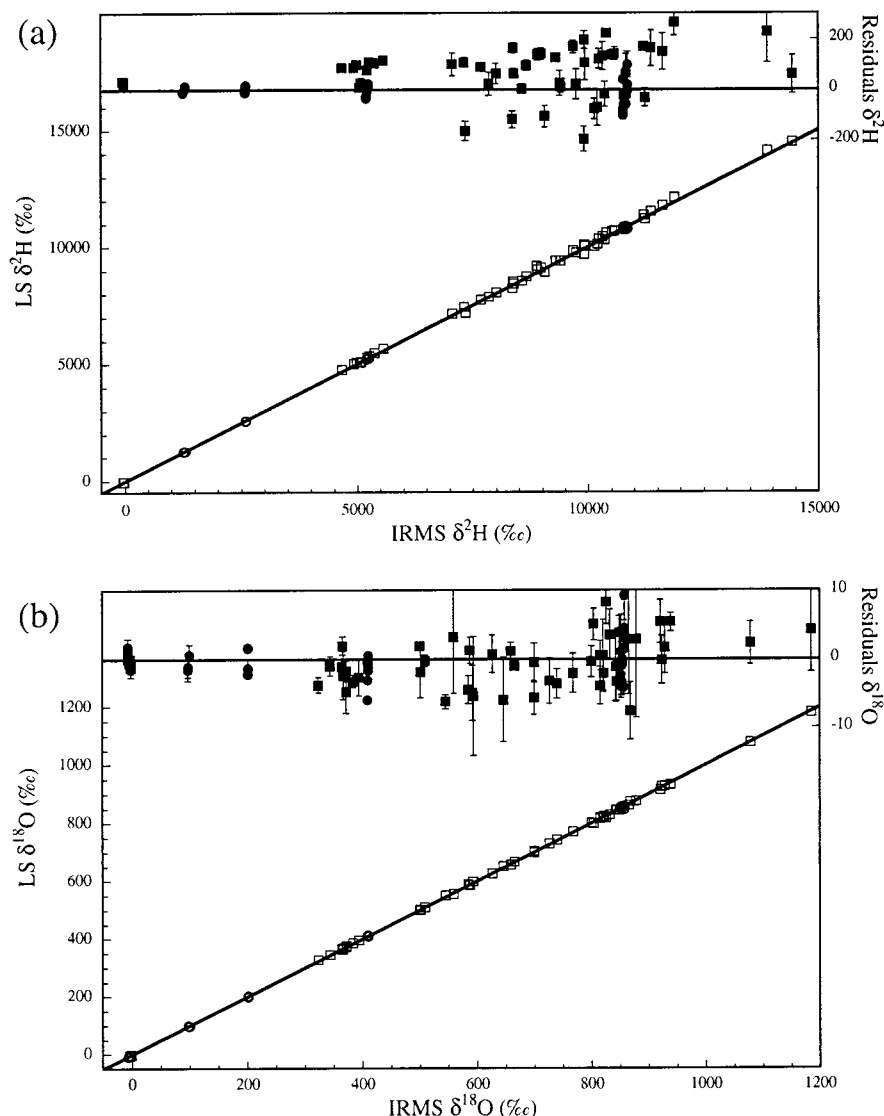


Figure 3. (a) $\delta^2\text{H}$ and (b) $\delta^{18}\text{O}$ values of all LS measurements vs the corresponding IRMS values as well as their differences (residuals). Circles represent the measurements of working standards; squares give the measurements of unknown samples. Each point represents the mean of repeated runs (LS, 5; IRMS, 4) involving the same sample, the error bar gives the corresponding standard deviation, and the solid line represents the line with unity slope ($y = x$).

to a triply labeled water (TLW) method. The idea is to use the known difference in fractionation behavior between ^{17}O and ^{18}O to estimate the fractional water turnover by means of evaporation (as opposed to water loss due to, e.g., urine). This has been shown to work with tritium as the third isotope, but this has not found widespread acceptance because of the radioactive nature of this isotope.³³ Unfortunately, however, we estimate that the required accuracy of the oxygen isotope measurements is almost 1 order of magnitude beyond our current level.

Although the memory effect of the LS method is smaller than that encountered with H_2 gas production by reduction of water over uranium, as used in our IRMS laboratory, it is still limiting the ultimate accuracy for $\delta^2\text{H}$, as well as $\delta^{18}\text{O}$, measurements, especially at high enrichment levels. We expect that this effect can be reduced dramatically by moderate heating of the gas cells (up to 40°C or 60°C). We are currently making preparations to do so.

The sample throughput can be further improved by automation of water injection and evacuation sequence or by increasing the number of gas cells. The laser provides enough power to add many more cells, and this is relatively cheap when compared to the costs of an IRMS system.

The only preparatory step used is the distillation of blood samples prior to measurement. In the IRMS sample preparation system, this is usually done in an on-line setup, which can easily be connected to our gas cells, as well. That would eliminate the extra labor of off-line distillation and a possible source of errors. The degree of enrichment that can be measured with the LS method for ^2H is currently limited to about $15\,000\text{‰}$. In biomedical experiments on small animals exhibiting very high water turnover rates, initial enrichments for deuterium of up to $50\,000\text{‰}$ are sometimes encountered. With so much ^2HOH present in the gas cell, the absorption of the corresponding transition will make the sample optically practically black, leading to a serious decrease in accuracy of the $^2\text{H}/^1\text{H}$ isotope ratio determination. However, by switching to a nearby and much weaker ^2HOH absorption, we

(33) Haggarty, P.; McGaw, B. A.; Franklin, M. F. *J. Theor. Biol.* **1988**, *134*, 291–308.

should be able to extend our measurement range upward to values satisfying biomedical requirements in all cases and with acceptable accuracy.

The most fundamental improvement would be the replacement of the FCL laser system with a diode laser. This would not only have technical advantages, which would be expected to lead to improved precision and higher sample throughput, but would also result in a more compact and cheaper apparatus. We are currently investigating the possibilities of using such a diode laser.

CONCLUSIONS

The LS system is a reliable tool for measuring the stable isotopes in water from biomedical applications in a wide range from natural up to 10 500‰ for $\delta^2\text{H}$, 1200‰ for $\delta^{18}\text{O}$, and 350‰ for $\delta^{17}\text{O}$. The accuracy and precision of isotope ratio determinations with LS are comparable to those of IRMS for $\delta^{18}\text{O}$ and are clearly better for $\delta^2\text{H}$. Sample throughput of the LS apparatus (30–40 measurements/day) is comparable to that of our IRMS laboratory but can be increased easily and at moderate cost. The biggest advantage of the new system is its conceptual simplicity

and the absence of chemical sample pretreatments that are necessary with the traditional IRMS method. Also new is the possibility of measuring ^{17}O , which conceivably may be used in a triply labeled water method once further improvements in accuracy have been made.

ACKNOWLEDGMENT

We thank Berthe Verstappen-Dumoulin and Trea Dijkstra for performing the distillations and IRMS sample preparations, Jaap van der Ploeg for his excellent technical support, and Mairead McLean and Karen Krijgsveld for collecting some of the blood samples. We are grateful to Stichting FOM and the CIO Research Fund for their financial support. E.R.Th.K. thanks the Royal Dutch Academy of Sciences for a 5-year fellowship.

Received for review December 5, 2000. Accepted March 14, 2001.

AC001428J